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Structure and Dynamics of Angiotensin (1-7) Vasoactive Peptide in Aqueous Solution at the Density-Functional Based Tight-Binding Level

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Summary: Structure and dynamics of heptapeptide Angiotensin (1-7) in aqueous solution have been investigated by means of density-functional based tight-binding molecular dynamics simulations. Solvent-solute interactions have been studied using a hybrid QM/MM method. The backbone of the heptapeptide remains relatively rigid in aqueous solution compared to gas phase. The solvent acts as a cushion, preventing the free motion of the molecule. Tyrosine is the residue which presents the smallest flexibility and the largest number of water molecules in its first solvation shell. This is in good agreement with the previously published NMR results. The intra– and intermolecular hydrogen bridges have been quantified and analyzed in terms of conformation and stability.

Keywords: Angiotensine-(1-7); DFTB; molecular dynamics

Introduction

The biologically active heptapeptide angiotensin (1-7), ang-(1-7) – (1) (Figure 1) inhibits vascular smooth muscle cell growth and contributes to the regulation of blood pressure. Ang-(1-7) belongs to the angiotensin family of peptides, and evidence suggests that it could be used to treat cardiovascular diseases.[1,2]

Recently, inclusion compounds of Ang-(1-7) and β-Cyclodextrines (CD) have been proposed as a formulation to overcome the problem of degradation of the drug due to the digestive enzymes of the stomach.[3] We recently studied the heptapeptide angiotensin (1-7) with the amino acid sequence AspArgValTyrIleHisPro and its β-cyclodextrin inclusion compounds by employing different physical-chemical techniques and the complete attribution of their NMR signal.[3]

The dispersion corrected self-consistent charge density-functional based tight-binding (DC-SCC-DFTB)/universal force field (UFF) hybrid method has been shown to be suitable for the structure and dynamic analysis of macromolecules in aqueous solution.[4] The flexibility of the backbone and the different residues of the heptapeptide ang-(1-7) is important for the understanding the chemical behavior of this molecule in solution and its interaction with cyclodextrines. The solvent water has an important role in stabilizing the different conformations.

The proposal of this paper is to analyze the structure and the dynamics of ang-(1-7) at aqueous solution using the DC-SCC-DFTB/UFF method. As a compromise between a first-principle method and an empirical force field we decided to employ a hybrid quantum mechanics/molecular mechanics (QM/MM) method,[5] which treats the solvent using molecular mechanics (MM) and the polypeptide quantum mechanically. This approach has been used with remarkable success to study the dynamics of β-cyclodextrine in solution.[4] Density-functional based tight-binding

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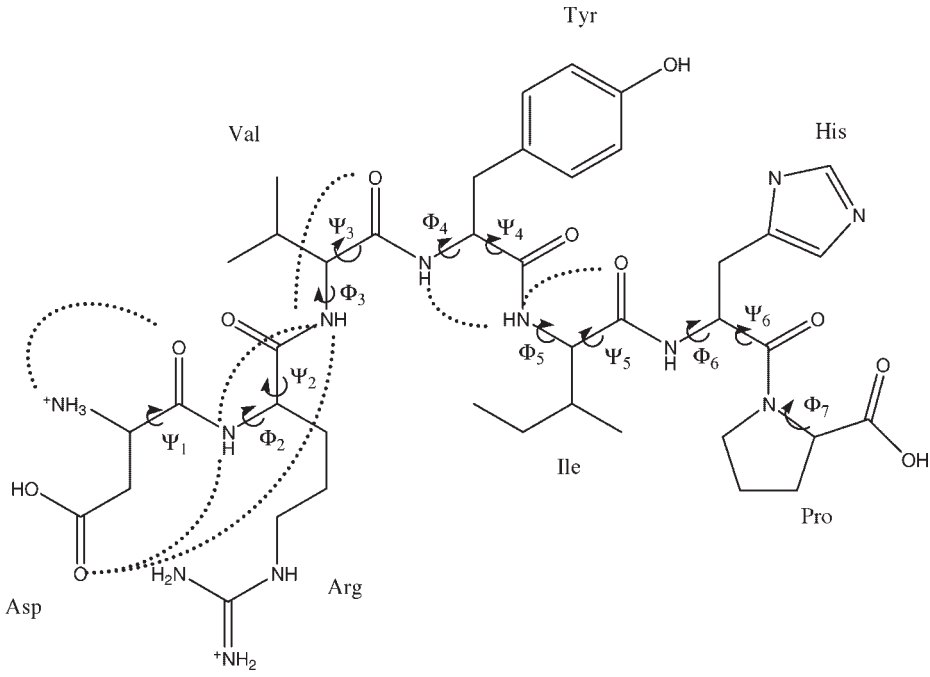


Figure 1.
The chemical structure of angiotensin (1-7), [AspArgValTyrIleHisPro]. Dashed lines show the intramolecular HBs present during the simulation of Ang-(1-7) in aqueous solution. See text for detail.

(DFTB) method with self-consistent charge (SCC)[6] and London dispersion corrections (DC)[7] has been used for the QM part and Universal Force Field (UFF)[8] for the solvent molecules permitting simulations to ns time scale.

Computational Aspects

All implementations and calculations have been performed with the experimental version of the deMon code, which is available free of charge for personal and academic uses.[9] For QM/MM calculations, the QM part has been treated with the DFTB method[10,11] including the second-order density correction scheme (self-consistent charge, SCC),[6] and the correction for London dispersion (dispersion correction, DC)[7,12] as implemented in deMon (DC-SCC-DFTB). The SCC-DFTB method has been thoroughly tested for biological molecules by Elstner and coworkers,[13,14] and hybrid calculations involving SCC-DFTB for systems of biological interest have been reported earlier.[15,16] The molecular mechanics part employs Rappé's universal force field (UFF),[8] with the partial charges taken from the TIP3P interaction potential for water.[17] The chosen MM scheme yields a self-diffusion coefficient of water of 3.37 10^{-5} cm^2/s at ambient conditions, which is higher than in experiment,[18] but still in closer agreement than TIP-3P-based force fields.[19]

The employed QM/MM[5] subtraction scheme with electrostatic embedding restricts the force field to act on the solvent and on the solvent-solute interactions. To account for the latter ones, fixed charges are put to the β-CD molecule. The MM part of the simulation is carried out within periodic boundary conditions (PBC). Here, the Coulomb interactions are calculated using the Ewald technique and van-der- Waals interactions are computed within the basis box of the super cell applying the minimum image convention. For Ang- (1-7), the simulation box is cubic

with an adequate lattice vector length of 55.15 Å, including 5564 water molecules and solute. The solute is calculated as a gas-phase molecule using DC-SCC-DFTB.

For the Born-Oppenheimer molecular dynamics simulations the following protocol was followed: All trajectories have been carefully heated up and finally equilibrated for 20 ps using the Berendsen thermostat[20] with a coupling parameter of $\tau = 0.1 \ldots 1$ ps. For the production run of 80 ps, a time step of 0.5 fs was chosen. The microcanonical NVE ensemble (constant number of particles, volume and energy, that means, no energy transfer from or to the medium) was used during the production run. The total energy remained constant within 0.001 Hartree during the whole simulation and did not show a drift.

For the calculation of structure and dynamics, details on the calculation of radial functions are given in the respective sections.

Results and discussion

Angiotensine-(1-7) in Gas Phase

The Ang-(1-7) was optimized in the gas-phase as a benchmark for the DC-SCC-DFTB method. The optimized parameters of the Ang-(1-7) gas-phase structure are compared with the PBE/DFT calculation (Table 1). The backbone dihedral angles are well described compared to the PBE/DFT results. The largest difference is for the Ψ_3 that is related to valine residue. The differences in the backbone dihedral angles are not larger than 30 degrees.

Angiotensine-(1-7) in Aqueous Solution

All computations reported in this section were performed using a hybrid QM/MM technique, as described above. Figure 2 shows the configurational space of the ang-(1-7) during the simulation. The changes can be quantified by the root mean square deviations (RMSD) of the coordinates between two snapshots of a MD trajectory, as proposed by Lawtrakul et al.[21] To account for long-time dynamical effects of the Ang-(1-7), only heavy atoms are taken into account, and the time average over the trajectory is taken.

$$\langle RMSD \rangle = \frac{1}{N_{\{C,O\}}} \sqrt{\sum_{k \in \{C,O\}} \left\langle |\vec{r}_k(t) - \vec{r}_k(0)|^2 \right\rangle} \quad (1)$$

In eq. 1, N denotes the number of non-hydrogen atoms of Ang-(1-7). Figure 3 shows $\langle RMSD \rangle$, plotted against simulation time for Ang-(1-7) in gas phase and in solution. In solution, $\langle RMSD \rangle$ converges to an asymptotic value of approximately 1.5 Å. In gas phase the molecule completely changes its geometry during the simulation at 300 K, converging to the value around 4.5 Å at the DC-SCC-DFTB level of

Table 1.
Structural parameters calculated for Ang-(1-7) at the DC-SCC-DFTB and DFT/PBE levels of theory.

Angle	DC-SCC-DFTB	PBE/DZVP	DC-SCC-DFTB/MM MD
Ψ1 (asp-arg)	76.0	65.0	74 ± 129
Ψ2 (arg-val)	28.9	38.3	36 ± 66
Φ2 (arg-val)	−132.4	−148.0	−104 ± 117
Ψ3 (val-tyr)	109.5	72.4	77 ± 127
Φ3 (val-tyr)	−67.5	−80.7	−122 ± 55
Ψ4 (tyr-ile)	−42.5	−45.3	28 ± 63
Φ4 (tyr-ile)	−60.9	−64.5	−101 ± 19
Ψ5 (ile-his)	63.6	40.3	74 ± 126
Φ5 (ile-his)	27.3	53.2	−95 ± 47
Ψ6 (his-pro)	156.1	167.5	35 ± 56
Φ6 (his-pro)	60.1	65.2	25 ± 42
Φ7 (pro)	−89.3	−93.4	−73 ± 20

The angles are given in degrees (°). Hydrogen bond distances are in Ångstrom (Å). See Figure 1 for the definitions of structural parameters.

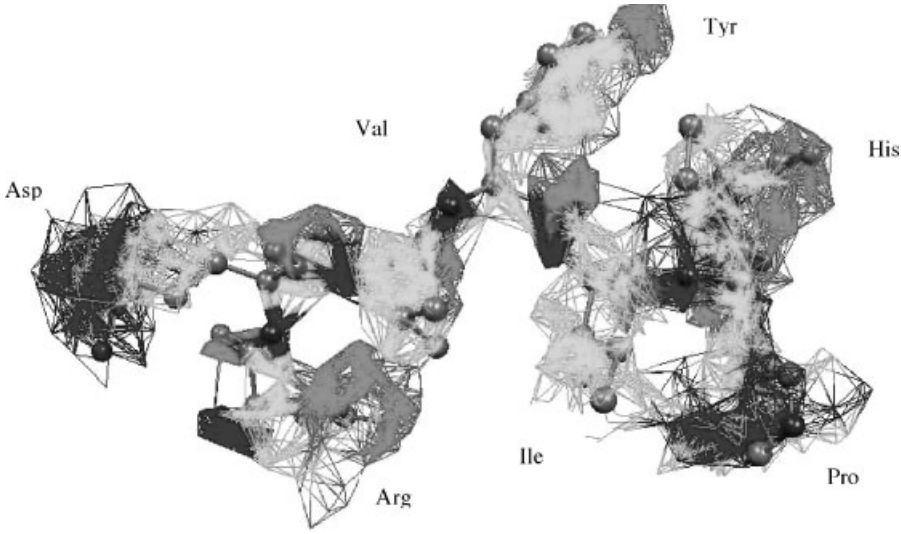


Figure 2.
Configurational space taken by Ang-(1-7) in aqueous solution.

theory. Actually, the water surrounding the Ang-(1-7) acts as a cushion, decreasing its free motion. As it has been pointed out elsewhere,[4] this effect cannot be seen in pure MM calculations using the UFF force field. The force field is restricted to the area close to the experimental structure of the typical peptide bonding leading to an excessively rigid polypeptide framework.

In Table 1, structural parameters of the optimized Ang-(1-7) are compared with those of Ang-(1-7) in aqueous solution. The latter ones are the time averages of the mean values, as discussed above. The differences with the gas phase DC-SCC-DFTB results are not larger than 60 degrees. The unique exception is the Φ_5 (ile-his) dihedral angle which is about 120 degrees

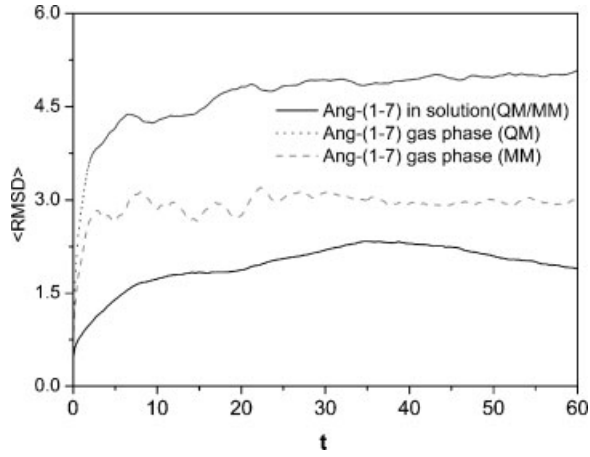


Figure 3.
Root-mean-square deviation, ⟨RMSD⟩, see eq 1, of the coordinates between a two snapshots of a MD trajectory (in Å) (gas phase in blue dotted line) against simulation time t (in ps). The simulation in solution is given as black solid line, the DC-SCC-DFTB and UFF gas-phase simulation as dotted (blue) line and, dashed (red) line respectively.

difference from the gas phase result. This is probably due to the tradeoff between the intra– and intermolecular hydrogen bonds. The backbone of the polypeptide is relatively rigid and the residues are flipping around the equilibrium geometry. The configurational space taken by Ang-(1-7) in aqueous solution is shown at Figure 2. The peptide dihedral angles – Ψ's and Φ's – have averages with large standard deviations of about 80 degrees. The Φ_4 dihedral angle which is related to the tyrosine motion presents the smallest standard deviation of about 20 degrees. Tyrosine is relatively rigid in comparison to the other residues along the simulation. This can have important consequences for the inclusion compound. Decrease of entropy is normally accompanied by the interaction of the guest:host inclusion compound due to the loss of flexibility. However, if the tyrosine is included, the loss of entropy is minimized. This is in agreement with the NMR based structure which shows small flexibility of the tyrosine and arginine residues.[3]

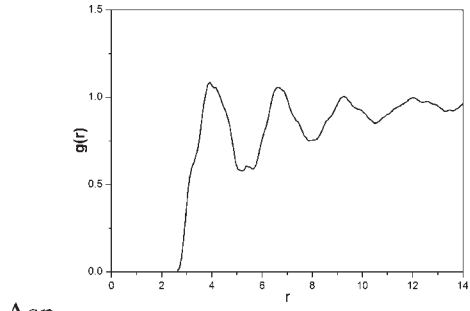
As a final structural property, we analyze the formation of hydrogen bonds (HBs) between Ang-(1-7) and water. Following Lawtrakul et al.,[21] we define the criterion for the existence of a hydrogen bond between donor (D) and acceptor (A) that (i) the D–A distance is less than the value corresponding to the first minimum of the respective D–A distance RDF, and (ii) the DH–A distance is less than 2.8 Å. Table 2 shows the average number of water molecules forming hydrogen bonds with the proton acceptor sites in each of the Ang-(1-7) residues. The average A–D bond distances are also shown. Arginine with its three proton acceptor sites has the largest average number of HB's, about 5.3 ± 0.9. Aspartate with its carboxyl group has about 4.8 ± 1.3 HB's, followed by histidine and tyrosine with 5.5 ± 1.0 and 3.0 ± 1.2 HB's, respectively. The terminal carboxylic and amine groups have an average number of HB's about 5.6 ± 1.1 and 2.6 ± 0.8, respectively. The intramolecular hydrogen bonds have also been estimated. The intramolecular HBs correspond about 25% of the total number of HB's. Figure 1 shows that most prominent intramolecular HB's in the Ang-(1-7), which are mostly involved with the aspartate and valine residues. It has also been observed intramolecular HBs in the peptide backbone.

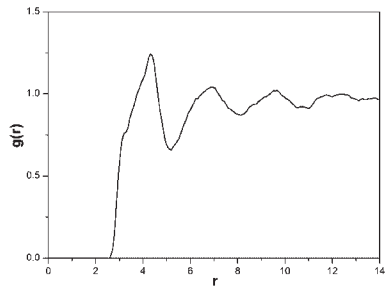
The radial distribution functions (RDFs) of the water surrounding the different residue for the Ang-(1-7) are shown at Figure 4. The RDFs are related to the distances of the residue center of mass and the H_2O. On average, the first solvation shell around the residues is about 6 Å from its center of mass with about 16 water molecules. The bulky tyrosine residue has in its first solvation sphere about 20 water molecules. The histidine residue has about

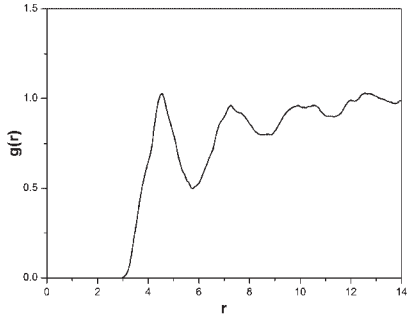
Table 2.
Average number of hydrogen bonds and corresponding donor-acceptor distances between water and ang-(1-7) residues.

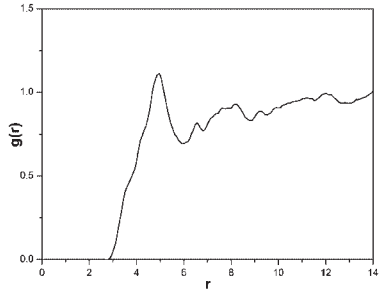
Residue	Average number of HB's		Bond distance (Å)	1st sphere radii (Å)	Number of water molecules
	With solvent	intramolecular			
Asp	4.8 ± 1.3	1.65 ± 0.70	3.14 ± 0.24	5.5	16
Arg	5.3 ± 0.9	5.05 ± 0.37	3.14 ± 0.33	5.2	15
Val	–		–	5.9	15
Tyr	3.0 ± 1.2	0.0 ± 0.0	3.15 ± 0.17	6.0	20
Ile	–		–	5.9	16
His	5.5 ± 1.0	0.29 ± 0.63	3.34 ± 0.31	5.8	18
Pro	–		–	5.6	12
Backbone[a)]	17.7 ± 1.6	4.5 ± 1.4	3.30 ± 0.20	–	–
NH_3^+	2.6 ± 0.8	0.87 ± 0.45	3.03 ± 0.18	4.1	7
COOH	5.6 ± 1.1	0.0 ± 0.0	3.13 ± 0.23	5.2	14

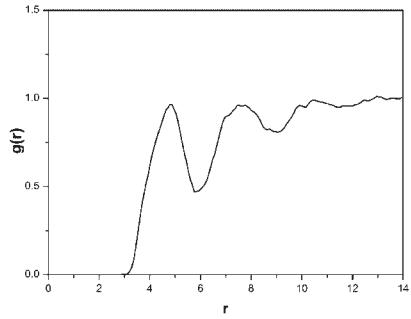
The first solvation sphere radii and the corresponding number of water molecules are also given.
[a)] The terminal groups $-NH_3^+$ and –COOH has not been taken into account.

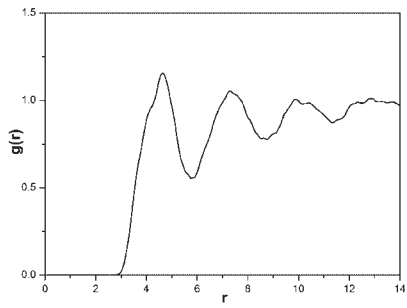












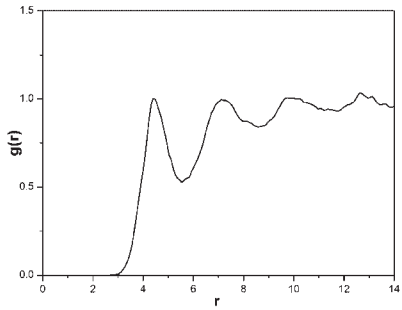


Figure 4.
Radial distribution functions, g(**r**), for the center of mass of the residue groups in Ang-(1-7) and centers of mass of the water molecules.

18 water molecules. The residues lacking of proton acceptor sites – Val, Ile, Pro – have less water molecules in the first solvation shell, as it is expected. The terminal groups $-NH_3^+$ and –COOH have about 7 and 14 water molecules in the first solvation shell, respectively.

Final Remarks

DC-SCC-DFTB/UFF hybrid method has been used to investigate Angiotensine-(1-7) in solution at the nanosecond time scale. In gas phase, Ang-(1-7) presents very large finite-temperature distortions from the ideal gas-phase geometry. In solution, the water acts as a cushion, restricting the motion of the solute to the vicinity of the $T = 0$ K structure. The residues are flipping around the minimum energy and the tyrosine presents the smallest flexibility in good agreement with the NMR results.[3] The average number of hydrogen bonds of each residue with the solvent water has been estimated and the results are in agreement with the expected tendency.

It is important to highlight that our results point out that the tyrosine residue presents the smallest flexibility and the largest number of water molecules in its first solvation shell. These results are coherent with the observation that tyrosine is the preferred residue to be included by β-cyclodextrin.[3] In the process of inclusion, normally the inclusion compound structure becomes more rigid, loosing entropy. However, tyrosine is more rigid preventing this lost of entropy through the inclusion process. On the other hand, the water molecules released from the first solvation shell of the tyrosine will contribute to the increase of entropy and, hence, contributing to the spontaneousness of the inclusion process.

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